PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
HMJ03637WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2004/003511	12.08.2004	12.08.2003

International Patent Classification (IPC) or national classification and IPC
C08B37/00, C07K17/12, A61K39/385, A61K47/48

Applicant
LIPOXEN TECHNOLOGIES LIMITED et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 49 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the opinion
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☒ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.03.2005	04.07.2005
Name and mailing address of the international preliminary examining authority:	Authorized Officer
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Form PCT/IPEA/409 (Cover Sheet) (January 2004)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003511

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-42	received on 14.03.2005 with letter of 10.03.2005
Claims, Numbers	
29-45	as originally filed
1-28	received on 14.03.2005 with letter of 10.03.2005
Drawings, Sheets	
1/23-4/23, 6/23-17/23, 19/23, 21/23-23/23	as originally filed
5/23, 18/23, 20/23	received on 14.03.2005 with letter of 10.03.2005

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☒ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-28
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-28
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-28
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item I
Basis of the report

The Applicant has replaced the feature "1-5 mL matrix" on original page 31, line 8, by "up to 75 mL matrix". The replacement of this feature introduces subject-matter which extends beyond the content of the application as filed, contrary to Article 19(2)/Article 34(2)(b) PCT.

The Applicant alleges that such a modification is in fact a correction of obvious error. However, for a modification to be considered as fulfilling the conditions for correction, it must be evident from the context of the application. This is not the case here.

Re Item IV
Lack of unity of invention

The objection of lack of unity no longer applies in view of the deletion of original claims 32-45.

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: EP-A-0 454 898 (SEIKAGAKU KOGYO CO LTD) 6 November 1991
D2: US-A-4 356 170 (JENNINGS HAROLD J ET AL) 26 October 1982
D3: US-A-5 097 020 (ANDERSON PORTER W ET AL) 17 March 1992
D4: GOUTAM SEN, CHITRA MANDAL: "The specificity of the binding site of Achatinin $_{H}$, a sialic acid-binding lectin from Achatina fulica" CARBOHYDRATE RESEARCH, vol. 268, 1995, pages 115-125, XP002303034

D1 is directed to glycosaminoglycan-modified proteins wherein the amino group of the protein is bound to an aldehyde group formed by:

- reducing and thereby cleaving the reducing terminal sugar moiety of the glycosaminoglycan which can be colominic acid with an alkali boron hydride such as sodium boron hydride and sodium boron cyanohydride,

- followed by partially oxidising the reducing terminal sugar moiety using alkali periodates such as sodium periodate or potassium periodate (see page 5, lines 22-39, and claim 7).

The aldehyde compound is then reacted with an amino group of a protein by reductive amination (see page 5, lines 40-46). Pharmaceutical compositions containing said glycosaminoglycan-modified proteins together with a pharmaceutically acceptable carrier or diluent are also described (claim 9).

In D2, the reducing end group of an antigenic polysaccharide is made into the most susceptible site for oxidation by initially reducing it to its open chain hydroxyl form, the terminal non-reducing sialic residues containing vicinal hydroxyl groups being then oxidated to yield a reactive aldehyde group which is then covalently linked to a free amino group of a selected protein by reductive amination (see column 3, lines 8-39, column 4, lines 27-44, and claims 1, 2, 4, 6-8 and 16). The antigenic polysaccharide can be derived from Meningococci and E. coli, Meningococcal group B polysaccharide being disclosed in example 1.

D3 relates to the formation of reducing groups on the capsular polysaccharide like Neisseria meningitidis serogroup C (see column 2, line 7) by selective hydrolysis, e.g. by acids, bases or enzymes, combined with a specific oxidative cleavage, e.g. by periodate or related oxygen acids (see column 3, lines 63-65) to form aldehyde groups via which the capsular polysaccharide can be covalently attached to bacterial toxins or toxoids by means of reductive amination (see column 4, lines 22-62).

D4 teaches that the oxidation of the trihydroxypropyl side chain of the sialic acid residue at the non-reducing end of the sialic acid-containing chain such as colominic acid, with periodate followed by borohydride treatment, i.e. reduction of the C-7 aldehyde group to a primary alcohol abolishes the inhibitory potency of said sialic acid compound towards the sialic acid binding lectin ATN_H.

1. Novelty - Article 33(2) PCT

1.1. The novelty of the subject-matter of present **claims 1-17** is acknowledged over D1-D4 since none of these documents discloses the preliminary passivation step a) of present

claim 1, resulting from the combination of original claim 1 and original claim 3.

1.2. The subject-matter of present **claims 18-26** (present claim 18 resulting from the combination of original claim 19 and original claim 20), as well as the subject-matter of present **claims 27 and 28** directed to compositions comprising a compound according to claims 18-26, are considered novel over D1-D4 because the claimed compounds differ from the known polysaccharides substituted with sialic acid in the presence of a passivated unit at the non-reducing end.

2. Inventive step - Article 33(3) PCT

The present invention is directed to the obtention of products of protein conjugation with PSAs, the polysialic acid being monofunctional i.e. activated at the reducing end with an aldehyde group and passivated at the non-reducing end, thus avoiding unintended by-products during conjugation by giving rise to single-orientation attachment to proteins and avoiding the need to purify away to obtain pharmaceutically-acceptable conjugates.

It follows that the steps of:

a) - selective oxidation at the non-reducing end of the PSA,
b) - reduction at both the reducing end and the modified non-reducing end,
c) - selective oxidation at the modified reducing end,

are essential to the obtention of a compound which can be easily fractionated by ion exchange chromatography.

D1 is regarded as being the closest prior art.

The subject-matter of claim 1 differs from this known process in that an additional step a) of oxidising the vicinal diol group at the non-reducing end of the sialic acid-containing chain is performed prior to steps b) and c).

The technical problem to be solved by the present invention may therefore be regarded as to provide a process for the provision of a monofunctional polysialic acid which can be fractionated by ion exchange chromatography.

The skilled person, face with this technical problem, would not have been prompted to combine the teachings of D1 and D4 to produce a monofunctional polysialic acid activated at the reducing end with an aldehyde group and passivated at the non-reducing end.

The procedure of D4 is applied to a glycoprotein, which does not have an available reducing end as it is the case for the compounds of D1, which document is concerned with chemistry relevant to the reducing end. Moreover, the present invention is based on the fact that the destruction of the potential of the non-reducing end for oxidation, as described in D4, can serve as part of the activation of the non-reducing end, which is not pointed at in the cited prior art.

The subject-matter of **claims 1-28** is therefore to be considered inventive.

3. Industrial applicability

The subject-matter of present **claims 1-28** appears to comply with the requirements of industrial applicability as stipulated in Article 33(4) PCT.